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COMPARITIVE STUDIES ON SPRAY-DRYING AND FREEZE-DRYING OF POMEGRANATE (PUNICA GRANATUM L.) JUICE FERMENTED WITH *L.ACIDOPHILUS*

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ABSTRACT

The effect of drying of probiotic pomegranate juice (PPJ) by spray drying and freeze drying and its subsequent storage characteristics were investigated. The spray drying was carried out in three different air inlet temperatures of 130° C, 140° C and 150° C and freeze drying was carried out at -40° C. The changes in the moisture content, bulk density, water activity, pH, acidity, solubility and survivability of the *L.acidophilus* MTCC 447 bacteria during the drying process and storage period were monitored. The results revealed that the survival of *L.acidophilus* MTCC 447 bacteria was higher in spray dried powder having lowest inlet temperature but it was much lower than the freeze dried powder. During the storage period of four weeks, the results revealed that the moisture content, bulk density, water activity and acidity were found to be increased while, survival of *L.acidophilus* MTCC 447 bacteria, pH, solubility and total anthocyanin content (TAC) content were decreased.

KEY WORDS:
Pomegranate, Spray drying, Freeze drying, *L.acidophilus*.

INTRODUCTION

The health benefits of certain foods have been investigated for many years. Development of foods that promote health and wellbeing is one of the key research priorities of the food industry (Klaenhammer and Kullen 1999). Probiotics are increasingly used as food supplements, due to mounting scientific evidences supporting the concept that the maintenance of a healthy gut microflora may provide protection

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against gastrointestinal disorder including infections and inflammatory syndromes of the bowel (Parvez et al. 2006; Nomato 2005; Shanahan 2002, 2004; Madden and Hunter, 2002).

Currently, probiotic products are usually marketed in the form of fermented milk and yogurt. However, lactose intolerance and the cholesterol content are two drawbacks related to their consumption. It has been suggested that fruit juices could serve as suitable media for cultivating probiotic bacteria (Mattila-Sandholm et al. 2002). Pomegranate (Punica granatum L.) juice has become more popular because of the attribution of important biological actions. The antioxidant capacity of pomegranate juice is greater than other fruit juice and beverages (Seeram et al., 2008). Pomegranate is known to have considerable health-promoting properties with antimicrobial, antifungal, antiviral, anticancer, antioxidant and anti-mutagenic effects (Negi et al., 2003).

L.acidophilus and related species exhibit probiotic effects and have been utilized widely in food processing. It colonizes the intestinal tracts of man and higher animals and suppresses pathogenic microorganisms (Brennan et al., 1983). In addition, L.acidophilus has also been reported to possess functional properties, including antitumor activity, hypocholesterolemic actions and the ability to synthesize various vitamins (Dash, 1996).

Drying of fruit juice produces a stable, easy-handling form of the juice powder that reconstitutes rapidly to a good quality product resembling the original juice as close as possible. Dried juice products are used mainly as convenience foods and have long storage shelf life at ambient temperatures (Chiou et al., 2006).

Methods of production of probiotic juice powders should be such that adequate numbers of viable probiotic bacteria are maintained in the dried powder following manufacture, and also retention/stability of probiotic properties should be ensured throughout the shelf-life of powder. Both freeze-drying and spray-drying can be used for manufacture of probiotic fruit juice powders on a large scale (Wang et al., 2004).

Freeze-drying has been used to manufacture probiotic powders for decades and is based upon sublimation, occurring in three phases; pre-freezing, primary, and secondary drying (Palmfeldt and Hagerdal, 2000). Spray drying can be used to convert juices into stable powders with new possibilities for industrial applications. This technique has been widely used in the food and pharmaceutical industries. The evaporative cooling effect in spray drying and short contact times (5–100 s) allow some properties of foods such as nutrients, flavor, and color to be retained to a great extent (Troung et al., 2005). The objective of the present investigation was to study the physico-chemical and microbial properties of freeze dried and spray dried probioticated pomegranate juice powder during storage period.

MATERIALS AND METHODS

The pomegranate fruit of Bhagwa (Kesar) cultivar was harvested from pomegranate orchard located at Aimangala, Chitradurga district of Karnataka, India. The fruits were transported to the laboratory soon after the harvest. Only the mature and well ripened fruits were selected and the fruits with defects were discarded. Probiotic bacterium (Lactobacillus acidophilus 447) was obtained from MTCC (Microbial Type Culture Collection), Institute of Microbial Technology, Chandigarh, India.

PREPARATION OF PROBIOTIC CULTURE

The probiotic ampoule procured was broken and by applying necessary microbiological measures, the cultures were cultivated using MRS broth (dextrose 20.0 g/l; meat peptone 10.0 g/l; beef extract 10.0 g/l; yeast extract 5.0 g/l; sodium acetate 5.0 g/l; disodium phosphate 2.0 g/l; ammonium citrate 2.0 g/l; tween80 1.0 g/l; magnesium sulphate 0.1 g/l, manganese sulphate 0.05 g/l) at 37° C for 36 h (Mestry et al., 2011).

FERMENTATION OF POMEGRANATE JUICE

The undiluted pomegranate juice with 16° Brix was autoclaved at 1.1 kg/cm2 for 15 mins. For obtaining an initial cell density of 107 CFU/ml in the final juice, 10 per cent of the cultivated MRS broth was centrifuged at 4000 rpm for 10 mins and the biomass was introduced into the juice. The juice was incubated at 37° C at 100 rpm in an orbital shaker (Remi).

JUICE POWDER PRODUCTION

The process flow chart of production of probiotic pomegranate juice powder using freeze drying and spray drying is presented in Figure 1.

PHYSICO-CHEMICAL AND MICROBIAL ANALYSIS

The moisture content of the probiotic fruit juice powder was determined by using a hot air oven as per the procedures outlined by AOAC (2005). The bulk density of the probiotic juice powder was determined by tapping method (Yousefi, et al., 2010). Two grams of powder was weighed into a 10 ml graduated cylinder. The cylinder containing the powder was tapped on a rubber mat to a constant volume from a height of 100 mm constantly. The solubility of probiotic pomegranate juice was determined according to the method described by Chauca et al. (2004). Water activity of probiotic pomegranate juice powder of 0.5 g was measured at room temperature using water activity meter (Rotronic AQUALAB series, Germany). The color of the probiotic pomegranate powder and reconstituted samples was analysed using Hunter's colorimeter (Hunter's Lab flex colorimeter) as per the method described by Yousefi et al. (2010). The pH was determined by using a digital pH meter (Systronics µpH 361 system, Ahmedabad, India). The titration method was used for determining the acidity level of pomegranate fruit juice was outlined by Zarei et al. (2010). The standard serial dilution spread plate method was adopted to evaluate the Lactobacillus acidophilus growth in powder as per the procedures outlined by AOAC (2005).

TOTAL ANTHOCYANIN CONTENT

The total anthocyanin content (TAC) of the pomegranate juice was determined using the pH differential method with two buffer systems by following the method outlined by Zarei et al. (2010). The potassium chloride buffer has pH of 1.0 (25 mM) and sodium acetate buffer had pH of 4.5 (0.4 M). The pH was attained by adding HCl.

Two grams of powder was dissolved in approximately 20 ml of distilled water and centrifuged at 4000 rpm for 15 min. The supernatant was used for the measurement. 1 ml of samples were mixed with 24 ml of corresponding buffers and read against water as a blank at 510 and 700 nm. Absorbance (A) was calculated by using the equation.

$$A=(A_{510}-A_{700})\ pH_{1.0}-(A_{510}-A_{700})\ pH_{4.5} \quad \ldots (1)$$

The total anthocyanin content (TAC) of each sample (mg cyanidin-3-glucoside/100 ml) was found out by using the equation.

$$TAC = \frac{A \times MW \times DF \times 100}{MA} \quad \ldots (2)$$

Where, MW is molecular weight of cyanidin-3- glucoside (449.2), DF is the dilution factor (25), and MA is the molar extinction coefficient of cyanidin-3-glucoside (26,900).

RECONSTITUTION OF PROBIOTIC POMEGRANATE JUICE POWDER

The powder was reconstituted at the rate of 16° Brix by slowly dissolving powder in 100 ml water to serve as ready-to-serve beverage. The quality parameters viz., pH, titrable acidity and total anthocyanin content (TAC) were analysed.

SHELF LIFE STUDIES OF PROBIOTIC POMEGRANATE JUICE POWDER

The freeze dried and spray dried probiotic pomegranate juice powder samples were packed in aluminium laminated foil (ALF) and hermetically sealed. The packed probiotic pomegranate juice powder was stored at room temperature for four weeks. The packed probiotic pomegranate juice powders were cut open at the intervals of every one week to assess the quality parameters and the same material was not used for the second week. The powder was reconstituted at the rate of 16° Brix by slowly dissolving powder in 100 ml water to serve as ready-to-serve beverage. The quality parameters viz., pH, titrable acidity and total anthocyanin content (TAC) were analysed after reconstitution only.

STATISTICAL ANALYSIS OF EXPERIMENTAL RESULTS

Analyses of variance (ANOVA) were conducted to determine whether significant effect existed on type of drying, packaging materials and storage period on the quality of probiotic fruit juice powder.

RESULTS AND DISCUSSION

The variation in moisture content of PPJ powder during storage is shown in Table I. There was a gradual increase in moisture content of the samples in Aluminium laminated package. It was observed that the initial moisture content of freeze dried powder is lower than the spray dried powder of 130 °C, 140 °C and 150 °C, respectively. It has been observed that there is higher absorption of moisture content in freeze dried powder. The combined effect of various operating parameters on moisture content showed that the increase in inlet air temperature resulted in a greater temperature gradient between atomized feed and the drying air, providing a higher driving force for moisture removal, resulting in lower residual moisture in the final productThe PPJ powder samples exhibited a caking tendency after 4th week. The samples therefore lost their free flowing characteristics and formed lumps.

The variation in bulk density of PPJ powder during storage is shown in Table I. The effect of drying rate on powder bulk density depends on its effect on moisture content due to the sticky nature of the product. The higher the powder moisture content, the more particles tend to stick together, leaving more interspaces between them and consequently resulting in a larger bulk volume. The initial and final bulk density during storage was ranged in between 0.327 to 0.453 g/cm3 and 0.573 to 0.709 g/cm3 for freeze and spray dried PPJ powder, respectively.

The effect of storage on water activity of PPJ powder was shown in Table I. The water activity indeed expresses the availability of water in the products and then its aptitude to take part in the reactions as solvent or reagent. It is then necessary to quantify this water activity of which the degradation speed of the products strongly depends (Schuck et al., 2004). The initial and final water activity during storage was ranged in between 0.203 to 0.352 and 0.319 to 0.437 for freeze and spray dried PPJ powder, respectively. It can be observed that the highest increase was recorded in the powder which had a lower initial water activity and lower increase was recorded by the sample which had the highest initial water activity.

The effect of storage on pH and titrable acidity of reconstituted probiotic pomegranate juices from freeze dried and spray dried powders are presented in Figure 2. Decrease in the pH values of the reconstituted juice was observed during the experimentation. This might be because of the increase in the acidity values of the juice. The increase in the acidity values of the juice might be due to the production of lactic acid by the L.acidophilus and utilization of sugars available in the powder by these organisms for their survival.

Effect of storage and drying on the solubility of the PPJ powder is shown in Figure 3. Initial solubility of the freeze dried and spray dried PPJ powder samples were 97.2, 95.2, 96.3 and 97.1 per cent, respectively. From this figure, it is noticed that the PPJ powder samples recorded a decrease in the solubility after four weeks of storage under ambient condition. Similar results were reported for spray dried mango milk powder by Sharma et al. (1974). The solubility of the powder is dependent on the additives also, as stated by Yousefi et al. (2010), the gum arabic recorded the best solubility results. There was no significant difference between the solubility per cent for maltodextrin and gum arabic.

SURVIVAL OF L.ACIDOPHILUS BACTERIA

The effect of freeze drying and spray drying on survival of L.acidophilus in probiotic pomegranate juice powder during storage is presented in Figure 4. The powder obtained by freeze drying showed the maximum survivability of L.acidophilus than the powder obtained using spray drying. The initial survival of L.acidophilus bacteria in freeze dried powder was 80 per cent. The spray dried powder resulted in 35, 28 and 21 per cent survivability of L.acidophilus bacteria in 130 °C, 140 °C and 150 °C, respectively. The decrease in the survival rate may be dependent on the water activity and the moisture content level of the powder during storage period. During the storage period, increase in the acidity and decrease in the pH and total sugars level of the powder was observed. This might be due to the production of lactic acid from the probiotic bacteria during the storage period of four weeks by consuming the sugars present in the pomegranate juice powder.

TOTAL ANTHOCYANIN CONTENT (TAC)

Effect of storage period of freeze dried and spray dried powder on total anthocyanin content (TAC) in the reconstituted PPJ is presented in Table I. The highest TAC was recorded in freeze dried reconstituted juice (24.56 mg/100ml), whereas, the lowest TAC was observed to be in spray dried reconstituted juice (19.17 mg/100ml) at temperature of 150 °C. The colour of pomegranate juice varied from light pink to violet, which was due to various anthocyanin pigments. The retention of anthocyanin is affected by temperature, Thus, variation in anthocyanin content of the samples might be related to the difference in the temperature of the spray drying and freeze drying for the production of the powder. Yousefi et al., (2010) stated that, during the spray drying, because of the higher stickiness of pomegranate juice samples, residues were collected from the connecting tubes, with greater air flow, which might be the cause of the lower total anthocyanin content.

CONCLUSION

Quality parameters of freeze dried probiotic pomegranate powder was better in terms of moisture content, solubility, bulk density, water activity and survivability of L.acidophilus than spray dried samples. The quality parameters included acidity, pH and total anthocyanin content (TAC). The acidity of the probiotic juice increased gradually, whereas, the pH and TAC of the freeze and spray dried samples were observed to decrease over the period of four weeks. Solubility of juice powders reduced gradually during the storage period of four weeks due to increase of bulk density of the juice powders.

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Figure 1 : Process flow chart for the production of PPJ powder

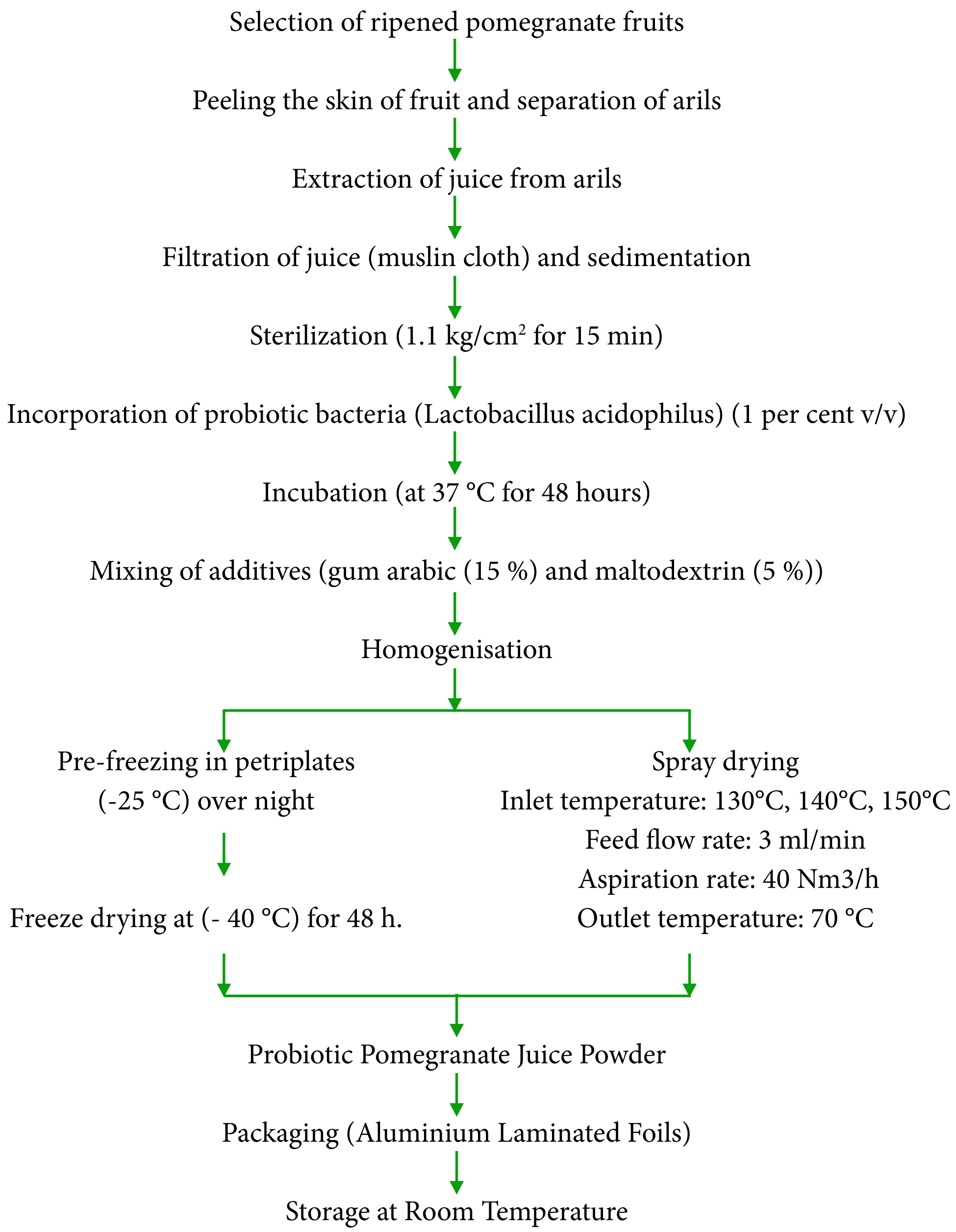

Figure 2 : of storage period on pH and titrable acidity of freeze dried (F-D) and Spray dried (S-D) PPJ powders

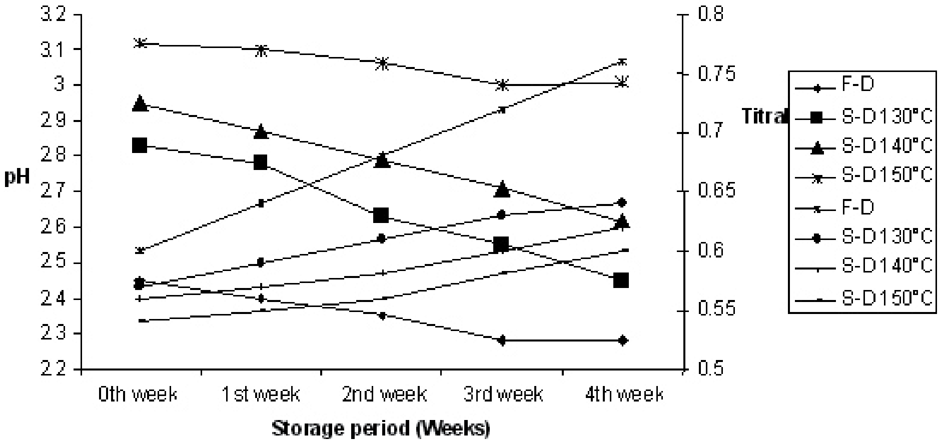

Figure 3 : Effect of storage period on solubility of freeze dried (F-D) and Spray dried (S-D) PPJ powders

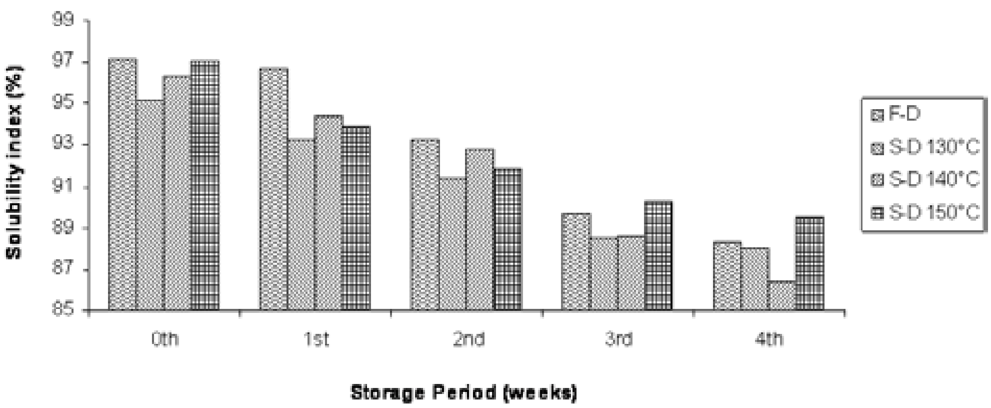

Figure 4 : Effect of storage period on survival of Lactobacillus acidophilus in freeze dried (F-D) and Spray dried (S-D) PPJ powders

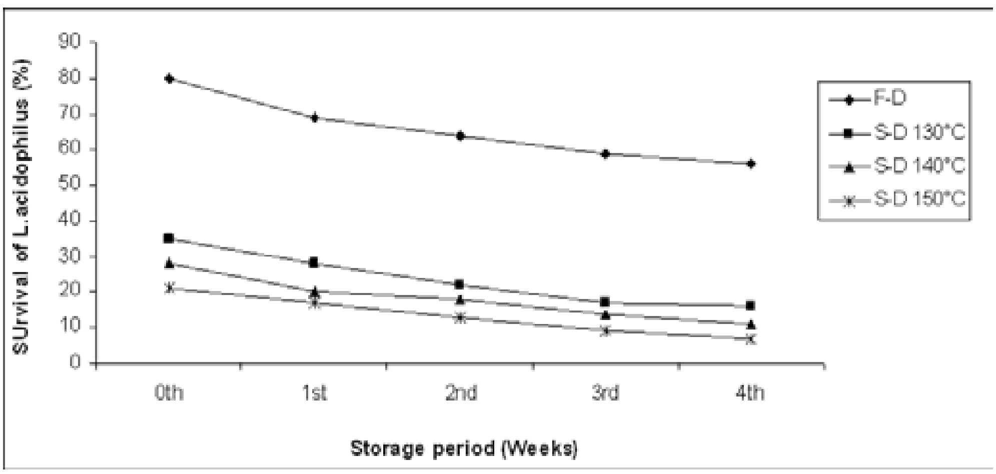

Table 1: Effect of storage period on moisture content, bulk density, water activity and TAC

Drying Condition	Moisture content (%) (d.b)					S.Ed.	CD (0.01)	CV (%)	'F' Value
	0^{th} week	1^{st} week	2^{nd} week	3^{rd} week	4^{th} week				
Freeze drying	2.01	2.88	4.00	5.27	6.53	1.3677	4.3348	40.89	0.9777
Spray drying at 130 °C	4.78	5.14	5.60	6.10	6.41	0.5320	1.6863	11.55	1.1592
Spray drying at 140 °C	4.08	4.62	5.20	5.80	6.01	0.6151	1.9494	14.65	1.1723
Spray drying at 150 °C	3.18	3.49	4.12	4.76	5.01	0.5641	1.7880	17.03	1.4987
Bulk density (g/cm3)									
Freeze drying	0.327	0.401	0.572	0.636	0.709	0.1188	0.3765	27.79	1.1458
Spray drying at 130 °C	0.453	0.498	0.546	0.607	0.657	0.0647	0.2050	14.25	1.0433
Spray drying at 140 °C	0.404	0.455	0.514	0.579	0.615	0.0660	0.2093	15.75	1.1585
Spray drying at 150 °C	0.350	0.407	0.463	0.514	0.573	0.0639	0.2025	17.18	1.3396
Water activity level									
Freeze drying	0.352	0.385	0.401	0.42	0.437	0.0241	0.0765	07.49	2.0664
Spray drying at 130 °C	0.243	0.281	0.319	0.347	0.371	0.0405	0.1285	15.80	0.9740
Spray drying at 140 °C	0.231	0.265	0.295	0.323	0.353	0.0370	0.1172	15.44	1.0751
Spray drying at 150 °C	0.203	0.243	0.273	0.291	0.319	0.0328	0.1040	15.32	1.3524
Total anthocyanin content (TAC) (mg/100 g)									
Freeze drying	24.56	23.32	22.28	19.47	16.67	2.6719	8.4683	15.55	0.5017
Spray drying at 130 °C	21.68	19.35	18.39	16.55	14.95	2.1149	6.7031	14.15	0.8347
Spray drying at 140 °C	20.65	19.33	17.63	15.25	12.03	2.7898	8.8420	20.12	0.6475
Spray drying at 150 °C	19.17	16.92	15.82	13.35	11.38	2.5135	7.9662	20.35	0.4964